Development of an Intermediate-Scale Aerobic Bioreactor to Regenerate Nutrients from Inedible Crop Residues

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SHAKER FLASK STUDIES

Preliminary decomposition studies were carried out, with replication, on a small scale to determine best levels of several key process variables. Experimental conditions were: 50 ml liquid working volume in 250 ml erlenmeyer flasks, 24°C incubation temperature, aeration by rotary shaker at 200 RPM, and soybean residue addition of 0.156 gdw day^{-1}. Key process variables were daily culture replacement rate (1/2 vs. 1/4 vs. 1/8) and soybean residue pretreatment (leached vs. unleached). The key response variable was percent weight loss of added crop residue. After 45 days, the best decomposition was a 38% weight loss for flasks receiving 1/8 daily culture replacement and unleached soybean residue.

It was observed that the pH of all treatments increased from 6.5 at day zero to 8.5 at day 45, so a follow-up study was designed to investigate the effects of pH control on crop residue decomposition. Replicate flasks were set up as before, but the de-ionized water was replaced with 0.1 molar phosphate buffer at pH 6.0 vs pH 8.0 in an attempt to control the pH. To determine if a decrease in the daily culture replacement might increase decomposition, 1/16 daily culture replacement was added to the treatments (and the 1/4 treatment was dropped). After 66 days incubation, decomposition in all treatments receiving pH 6 buffer exceeded the corresponding flasks receiving pH 8 buffer. Furthermore, little difference was observed between 1/8 and 1/16 daily culture replacement rates. Of note was that the 0.1 molar buffer concentration only partially controlled pH, as the pH 6 flasks ranged between pH 6.8 and 7.0 and the pH 8 flasks from 8.5 to 8.8.

Levels of other key bioreactor environmental parameters, i.e., incubation temperature (35°C), air flow (initially at 0.5 liters air per liter bioreactor liquid), and stirring rate (500 RPM), were selected from fermentation literature (5).

DEVELOPMENT OF AN INTERMEDIATE-SCALE AEROBIC BIOREACTOR (I-SAB)

Results from the shaker-flask studies indicated that aerobic processing of inedible plant biomass warranted further detailed investigation. However, the addition and harvesting of the solid substrate was found to be labor intensive and operator dependant. It was decided that subsequent studies should be carried out in well instrumented biological reactors. As evident in the shaker-flask studies, the handling and processing of the milled inedible biomass proved to be quite challenging. The prohibitive cost and marginal effectiveness of commercial bioreactors to adequately process the solids laden slurry resulted in a decision to design and construct a bioreactor in-house.

DESIGN OBJRCTIVES - At the start of the project a number of design objectives were established. The list included traditional goals as well as those specific to the processing of CELSS inedible biomass. The following section identifies the primary design objectives established for the development of an Intermediate-Scale Aerobic Bioreactor (I-SAB).

Solids Loading - Many industrial aerobic fermentation processes operate with solids loading as high as 50 g L^{-1}, however these high concentrations can only be maintained under special circumstances (6). Chemical anti-foams or mechanical foam breakers are often employed to control the foaming problems associated with these highly aerated systems (7). Additionally, the feed stream for many of these processes is soluble and the mixing problems associated with insoluble substrates is absent. Most waste-water treatment facilities maintain solids loading no higher than 5 g L^{-1} (6). Because of the low loading rate, these systems require minimal or no use of anti-foams and do not require high gas sparging rates. This is desirable since it is not likely that chemical anti-foams would be available in an operational CELSS and high gas sparging implies a high energy cost. In order to minimize fluid working volume, a high solids loading rate is preferred, however, the use of anti-foams or complex mechanical foam breakers is not. Therefor, the bioreactor was designed to handle a solids loading of 50 g L^{-1} or less, and do so without the aid of chemical anti-foams or a complex mechanical foam breaker.

Working Volume - The bioreactor should be capable of processing working volumes ranging between 6 and 12 L. This allows for a wide range in operational parameters such as the dilution rate (ratio of in-flow rate to working volume) and the overall production rate of the bioreactor. For example, given a typical dilution rate of 10 day^{-1}, the flow rate through the reactor would be 0.6 and 1.2 L day^{-1} for a 6 and 12 L working volume respectively. It was determined that these flow rates would be high enough to support future plant growth studies (8).

Mixing and Aeration - A primary design objective was that uniform mixing of the solid, liquid, and gas phases be achieved. Effective power input implies good mixing by the impeller and the gas sparger. It is important to ensure that the impeller Reynolds Number is high and therefore in the turbulent range. The oxygen mass transfer coefficient, k_La, is intimately tied to the mixing characteristics and must be maintained at a level that is not limiting to the biodegradation process. Typical values of k_La range between 3E-4 and 3E-3 s^{-1} for waste water treatment and up to 0.1 s^{-1} for bacteria and yeast fermentations (6). It has also been shown that the minimum dissolved oxygen concentration, above which O_2 is not limiting to microbial growth, ranges between 0.1 and 1.6 mg L^{-1} (7). Therefor, the aeration and mixing systems should sustain adequate values of k_La and maintain the DO above 1.6 mg L^{-1}.

Feeding and Harvesting - As previously discussed, feeding and harvesting during the shaker-flask studies were labor intensive and time consuming.

Since it was anticipated that I-SAB would be used in long duration studies (up to 6 months), it was important that these processes be improved.

Environmental Monitoring and Control - In addition to meeting aeration requirements, the I-SAB control system was designed to maintain temperature and pH at predetermined set-points. Since foaming could occur at the flow rate required to maintain proper DO, foam control was provided to prevent particle and liquid entrapment in the exiting gas stream. Controlled parameters such as stirring, feeding, and aeration rates, temperature, and pH as well as other primary process parameters such as CO_2 production and dissolved oxygen were continuously monitored and recorded.

I-SAB DESIGN

I-SAB was designed as a Continuously Stirred Tank Reactor (CSTR), a well documented design that has been used extensively in both research and industry. The traditional CSTR design includes a cylindrical mixing vessel with either a flat or domed bottom, a shaft mounted mixing impeller, and a gas sparger. CSTRs are adaptable to continuous culture and amenable to scale-up. More detailed analysis of CSTR design and operation can be found in references (5,6,7,9,10). Three I-SAB's were designed and fabricated between September, 1992 and January, 1993. The following section describes the various sub-systems of a complete I-SAB unit.

CONTAINMENT VESSEL - Each I-SAB vessel is 395 mm in height and 254 mm in diameter. For working volumes ranging between 6 and 12 L, the height/diameter ratio ranges between 0.5 and 1. Wetted materials of construction are stainless steel and lexan. For ease of fabrication a flat rather than domed bottom was constructed. Access ports are located at the 6, 8, 10, and 12 L levels of the vessel. Two large rectangular viewing ports allow visual inspection. Figure 1 shows a detailed view of I-SAB.

STIRRER ASSEMBLY - The stirrer assembly utilizes a sealed magnetic drive system which eliminates rotating seals and their corresponding complexity. A 40 W, variable speed gear motor (Model 104, Talboys) is attached to an outer rotor which is lined with permanent earth magnets. An inner magnet-lined rotor is separated from the outer rotor by a stainless steel cap. An O-Ring seated between the cap flange and the lid of the bioreactor creates an impermeable seal. A stainless steel shaft extends through a bearing block and into the vessel. A 51-mm diameter marine impeller is fixed on the end of the shaft and is located 51 mm above the tank bottom.

Maximum stirrer speed is 750 RPM with a nominal operating speed of 525 RPM. The impeller Reynolds Number (Re_i) is given by:
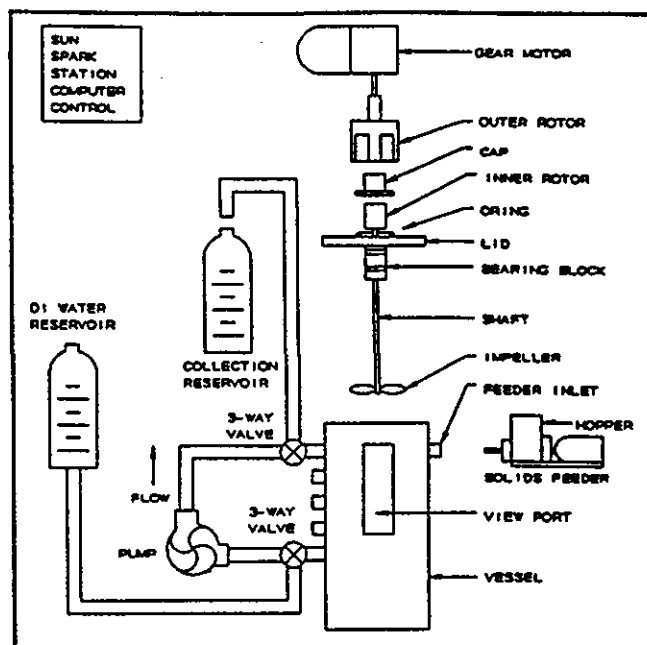
$$Re = \frac{N D^2}{v} \qquad (1)$$

Figure 1. Identification and configuration of I-SAB components.

where: N is the rotational speed
D is the diameter of the impeller
v is the kinematic viscosity of the fluid

Assuming the fluid is water ($v = $ 1E-6 m^2 s^{-1}), N = 525 RPM, D = 51 mm, and utilizing equation (1), Re_i for the stirrer assembly is calculated to be 2.3E4. This result confirms that the stirrer will operate in the turbulent region and therefor good mixing can be expected around the impeller.

Another technique used to assess mixing potential is to measure the power input of the stirrer into the system. Power input by an impeller (P_i) is given by (9):

$$P_I = N_P \rho N^3 D^5 \qquad (2)$$

where: N_p is the power number
ρ is the fluid density

N_p is a complex function of the geometry of a mixing system. For a baffled tank and marine impeller, Tatterson has estimated N_p to be 0.6 (9). For an unbaffled tank with a Re_i of 2.3E4 and using Tatterson's correlation, N_p was found to be 0.2. I-SAB does not utilize traditional baffles, however, the top mounted pH, dissolved oxygen, and temperature probes, and the gas sparger act like baffles and disrupt rotational flow. Therefor, for the purposes of this analysis a value of N_p = 0.4 was chosen. Assuming $\rho = 1$ kg m^{-3}, N = 525 RPM, D = 51 mm, and substituting into equation (2), P_i is found to be 9.24E-2 W. At a nominal working volume of 8 L the power to volume ratio, P/V, is 1.16E-2 W L^{-1}.

AERATION - Facility compressed air was utilized in the I-SAB aeration system. Coalescing air filters (Model DX and BX, Balston) remove 99.99% of

0.1 micron or larger particles and droplets. A thermal mass flow controller (Sierra Instruments) maintained the flow rate at a user defined set-point between 0 and 15 L min^{-1}. A 76-mm diameter ring sparger, located 25 mm off the bottom, aerates the bioreactor. Power input by the sparger (P_s) is given by (9):

$$P_s = Q\rho g H \qquad (3)$$

where: Q is the flow rate of the gas
g is the local gravitational level
H is the sparging height

The sparging height (distance between sparger and liquid surface) for a volume of 8 L is 142 mm. Substituting values of Q = 5 L min^{-1}, ρ = 1 g cm^3, g = 9.8 m s^{-2}, and H = 142 mm into equation (3) results in a P_s of 0.116 W. For an 8-L working volume, P_s/V is calculated to be 1.45E-2 W L^{-1}.

SOLIDS FEEDER - Ground inedible biomass (1 - 2 mm particle size) was introduced into the bioreactor by an auger feeder. The biomass was placed in a 400 cm^3, stainless steel hopper. Once the lid was in place, the hopper was sealed to ensure that no moisture laden air back-flows into the hopper from the bioreactor, as this would have led to clumping of the biomass and incomplete feeding. A motor driven, auger-type screw located in the bottom of the hopper conveyed solids out of the hopper and into the bioreactor, just above the liquid level. A 1600 RPM bin vibrator (Model SPR-20, Vibco) prevented bridging of the solids and assured a uniform flow. The volumetric flow rate for dried wheat inedible biomass could be varied between 10 and 30 cm^3 hr^{-1}.

HARVESTER ASSEMBLY -- Harvesting was accomplished by circulating bioreactor contents through an external loop and back into the bioreactor. A centrifugal pump (Model AC-4C-MD, March Manufacturing) moved the slurry through the loop at 5 L min^{-1}. Two 3-way valves were used to harvest the slurry and add deionized water. As shown in Figure 1, a 3-way valve, located just before the return port, was used to divert a part of the flow to a collection reservoir during harvest. This system assured uniform mixing and provided repeatable representative sample collection. After harvesting was complete, deionized water was added via the second 3-way valve and circulated through the external loop to flush remaining solids into the bioreactor and replenish the liquid volume.

pH AND TEMPERATURE CONTROL - Hydrogen ion concentration was maintained by injecting a 1N nitric acid solution into the bioreactor when the pH rose above a user defined set-point. A combination type pH electrode and transmitter (Model PHE-6510 and PHTX-92, Omega Engineering) continuously monitored pH in the bioreactor. Acid was pumped out of a graduated reservoir by a metering pump (Model 210-5R, March Manufacturing), and through a solenoid valve and

added through the bioreactor's lid. A 0.5-mm diameter nozzle located on the acid addition inlet produced a thin, high pressure stream of acid directed at the liquid surface. The jetting action of the stream penetrated the foam layer and improved dispersion of the acid within the bioreactor.

Temperature control was maintained by an external heating jacket. Heated water from a circulating water bath (Model 2095, Forma Scientific) was pumped through copper coils which were soldered to the external wall of the bioreractor. An insulation jacket comprised of 25.4-mm closed-cell foam insulation encased the bioreactor vessel and the heating coils. A thermocouple probe measured temperature inside the bioreactor. Temperature control was effected by regulating the flow of water through the heating coils with a computer controlled solenoid valve.

ENVIRONMENTAL MONITORING AND CONTROL - As previously discussed, pH, temperature and aeration mass flow were monitored and controlled. Additionally, a dissolved oxygen probe and transmitter (Model 4, Oxyguard) continuously monitored DO in the bioreactor. Also, bioreactor headspace gas was circulated through a carbon dioxide analyzer (Model LI-6251, LI-COR) to obtain the CO_2 concentration of the exiting gas stream.

Hardware for monitoring and control included a SUN Sparc Station and OPTO-22 digital and analog input/output boards. Software developed in-house (UNDACE V1.9), was used for monitoring and control and acted as the primary information interface between the operator and I-SAB. All monitored parameters were permanently recorded at 5 minute intervals on a Hewlett Packard Model 9000 I-50 mainframe computer. For more information on computer monitoring and control refer to reference (11).

RESULTS AND DISCUSSION

TEST RESULTS - I-SAB testing was conducted during the month of January, 1993. Testing included validation of the various pumps, valves, seals, and stirring motors. The harvest and water addition system and the solids feeder were also tested to ensure proper operation during experimental runs. Mixing time was measured with a working volume of 8 L (deionized water), aeration flow of 5 L min^{-1}, and a stirrer speed of 525 RPM. After initially adjusting the pH to 6.0, 3 mL of 1N HNO_3 was added to the bioreactor. The pH stabilized at 2.4 within 35 s.

The volumetric oxygen mass transfer coefficient (k_La) was measured using the static gassing-out method (12). I-SAB 3 was filled with 8 L DI water and continuously sparged with nitrogen to remove all dissolved oxygen. It was determined that measurement of k_La in DI water was sufficient since the viscosity and density of the actual slurry were approximately equal to that of water (ρ = 1.006 g cm^{-3} v = 10 cp). After sparging with N_2, stirrer speed and

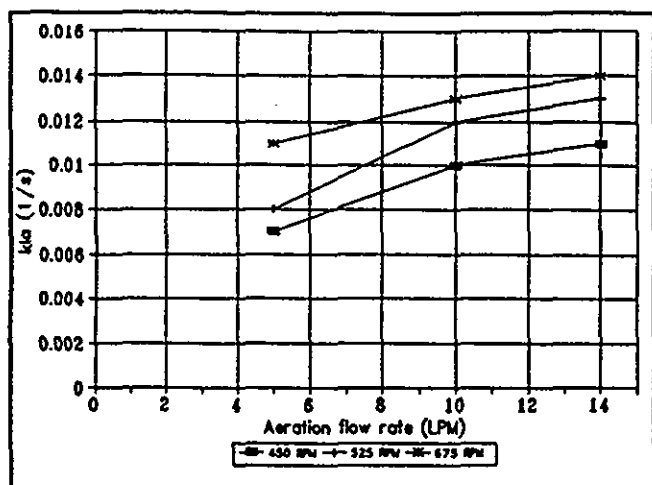Figure 2. Plot of k_La verses aeration flow rate for three different stirrer speeds.
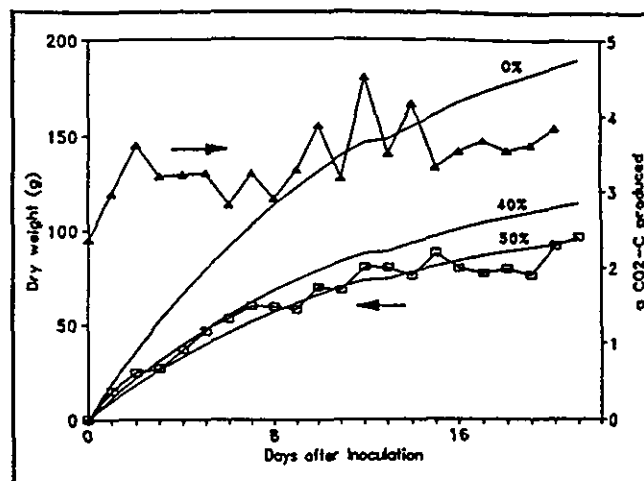

Figure 3. The upper plot shows daily CO_2 production, the lower plot shows the time-course of microbial decomposition of crop residues, and the solid curves represent theoretical decomposition of 0, 40, and 50%.

aeration were started at pre-selected values. Immediately after the start of aeration, the DO concentration was monitored and recorded until a steady-state concentration was achieved. DO-time profiles were recorded for 9 combinations of stirrer speed and aeration. The time course of DO after the start of aeration is given by:

$$\ln(1 - \frac{c}{c^*}) = k_La \, t$$

where: c is the instantaneous DO concentration
c* is the saturation DO concentration
t is time

The slope of the line generated by plotting $\ln(1-c/c^*)$ verses t, is equal to k_La. The results of the 9 runs are shown in Figure 2. Values of k_La ranged between 0.007 and 0.014 s^{-1} These values fall within the range reported for waste water treatment (6).

Temperature control was found to be quite stable and was routinely maintained at +/- 0.2 °C. Occasionally the water level in the hot water circulating bath dropped below a minimum requirement for continuous circulation. This resulted in transient lower bioreactor temperatures, but was found to have no long-term effects on bioreactor operation.

Generally, pH control was maintained at +/- 0.1 pH units throughout each operation. However, spurious electrical noise generated by the stirrer motor occasionally corrupted the pH transmitter signal and impeded control. Replacement of the stirrer motor brushes or stretching of their compression springs alleviated the problem.

EXPERIMENTAL RESULTS - A series of eight short I-SAB runs were used to determine the effects of three key process variables on response variables. Process variables were 1/4 vs. 1/8 culture volume replacement day[-1], 20 vs 40 gdw wheat residue addition day[-1], and with/without solids recycling (about1/3 of harvested solids were returned to the bioreactor). Response variables were dry weight loss and CO_2 production. Run durations were 14 days for 1/4 culture replacement and 21 days for 1/8 culture replacement.

Figure 3 shows the time-course of microbial decomposition of added crop residues during one of the eight bioreactor runs. Three theoretical lines are also plotted. The line labeled 0% represents dry weight of bioreactor contents if no decomposition occurred, while the lines labeled 40 and 50% represent dry weights if these amounts of the added biomass were decomposed, or lost. The plot of observed dry weight ranges between these two theoretical lines for most of the 21 day run. At harvest, 48% of added biomass had been decomposed, presumably to carbon dioxide.

Decomposition has been underestimated by dry weight loss because soluble minerals, released from the crop residue into the liquid, are included. Future studies will compare the weight loss of volatile solids (i.e., ash free dry weight) to correct for this underestimation. The dry weight measurements further underestimate decomposition because they include both undigested crop residues and microbial biomass. Another analytical problem has been finding a method to independantly determine the mass of crop residues and microbial biomass. Research is in progress to solve this problem for such a complex substrate.

The dry weight losses for all eight experimental bioreactor runs are shown in Table I. In general, decomposition of wheat residues was better at the lower daily culture replacement rate tested, 1/8 day^{-1} and lower residue addition, 20 gdw day[-1]. Solids recycling seemed to also provide for greater decomposition, but dry weight losses were not as dramatically different as for the other two process parameters.

A typical plot of daily CO_2 production and dissolved oxygen concentration during one of the

Table I. Comparison of effects of process parameters on bioreactor response variables of dry weight loss and organic carbon mineralization (i.e. CO_2 production).				
Culture Replacement	gdw added per day	Solids Recycled	Weight Loss (%)	Carbon to CO_2-C (%)
1/8	20	N	44	48
		Y	50	47
	40	N	40	29
		Y	41	28
1/4	20	N	31	47
		Y	34	41
	40	N	30	23
		Y	34	26

bioreactor runs is shown in Figure 4. When the dried wheat residue was added at about 08:30 there was an immediate stimulation of carbon dioxide production from just over 3 ml min⁻¹ to about 14 ml min⁻¹ with a concomitant decrease in dissolved oxygen from 5.5 to 1.5 mg L⁻¹. The most likely reason for this response was rapid microbial degradation of soluble organic compounds released from the crop residue. With time, both carbon dioxide production and dissolved oxygen returned to more stable levels.

The rapid decrease in dissolved oxygen was undesirable. Under anaerobic or anoxic conditions, microbial production of potential phytotoxic compounds (e.g., low molecular weight organic acids) can occur. Second, anaerobic conditions also favor microbial denitrification, or conversion of inorganic nitrate into nitrogen gas. The problem was solved in subsequent bioreactor runs by adding solids over a two to three hour period instead of all at once. To perform this task, a solids addition hopper with an auger located in the bottom was designed, fabricated, and installed on the outside of the bioreactor, so solids entered about 150 mm above the liquid level.

The daily carbon dioxide data, as shown in Figure 4, was used to calculate the sum of CO_2 produced each day. These summations for one of the runs are plotted in Figure 3, along with the corresponding dry weights taken at the same time. For this run, daily CO_2 production was stable from the start, with a slight increase from 2.9 at the beginning to 3.8 g CO_2-C per day at harvest. CO_2 production during the first seven days was higher than anticipated and does not correspond to the slower build up of dry weight losses. The soluble organic compounds that are "leached" from the crop residues are more easily and rapidly degraded than the cellulose and hemicellulose structural polysaccharides that account for ca. 50% of BPC wheat crop residues (1). The high CO_2 production rates early in each run probably reflect the rapid growth and degradation of soluble organic compounds,
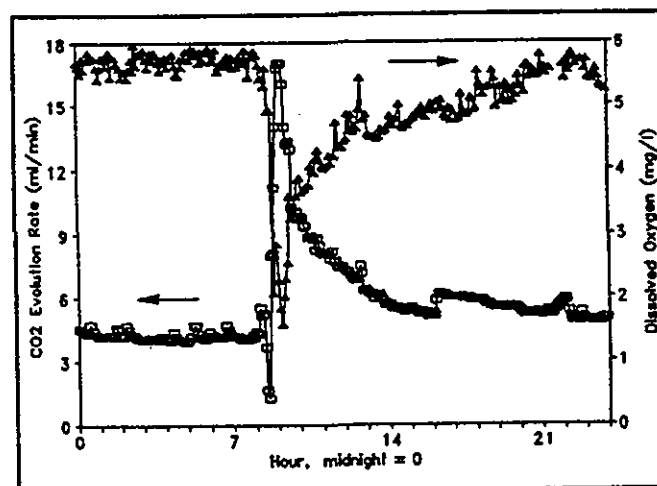

Figure 4. Typical plots of CO_2 production and dissolved oxygen concentration for a 24 hour period.

whereas the steady increase in CO_2 production rates reflect the buildup of slower growing microbes that degrade plant cellulose and hemicellulose.

For comparison with dry weight losses, the total amount of carbon dioxide produced during each run was calculated and converted into a percentage of the total carbon added as residue. Dreschel (13) reported that BPC grown wheat contained ca. 40% carbon (ultimate analysis), so this conversion factor was used. In further support for using this conversion factor, most of the crop residue was carbohydrate, which contain from 40 to 45% carbon, depending on the degree of polymerization. Total CO_2 production values are listed in Table 1. With carbon mineralization as the response variable, the lower solids addition rate of 20 gdw day⁻¹ gave highest conversion values. The lower culture replacement rate was slightly better than the higher one of 1/4 volume per day. Surprisingly, solids recycling did little to stimulate carbon conversion.

Table II. Comparison of mineral content of hydroponic replenishment solutions. Bioreactor effluent was the daily liquid harvest after removal of settled solids from I-SAB 1 which was fed BWT931 wheat residues. Leachate was made by soaking BWT931 wheat for 2 hours, after which the solids were removed. Hoagland's was the standard nutrient solution used at KSC for replenishment (8).

| | Bioreactor | | | Effluent as Percent | |
Component	Effluent (ppm)	Leachate (ppm)	Hoagland (ppm)	Leachate (%)	Hoagland (%)
NO_3-N	386	266	1050	145	37
PO_4-P	40	42	233	94	17
K	1618	1927	2652	84	61
Ca	145	157	300	92	48
Mg	102	121	238	84	43
Fe	0.71	2.66	11.1	27	6
Cu	0.57	0.20	0.6	285	95
Mn	1.38	1.49	3.73	93	37
Zn	0.63	0.20	3.73	315	17
Mo	<0.01	<0.01	0.94	-	0
B	1.31	1.91	0.94	69	139
CO_2-C	YES	NO	NO		
microbial biomass	YES	NO	NO		

The short duration of these initial I-SAB runs was inadequate to support tests of the ability of bioreactor effluent to regenerate plant nutrient solutions. A wheat production study was designed by KSC crop scientists to compare regenerated wheat hydroponic solutions with Hoagland's solution. Three different sources of solution were tested: A standard Hoagland's solution (control), leachate from BWT931 wheat residues, and effluent from bioreactors fedBWT931 wheat residues. Details of this study are being published elsewhere (8). The intermediate-scale aerobic bioreactor was used with 1/8 culture replacement, 40 gdw residue added per day, and no solids recycling. Environmental conditions were the same as for the earlier runs: pH 6.5, temperature 35°C, air flow 5 L min^{-1}. The duration of this run was 76 days. At day 23, the rapid vegetative growth of the test plants out-paced the ability of I-SAB #1 to supply effluent so I-SAB #2 was started with a 50% inoculum from I-SAB #1. I-SAB #2 ran for 47 days.

Response variables for the bioreactors were CO_2 production and inorganic content of the bioreactor effluent. Figure 5 shows production of CO_2 by both bioreactors. Microbial decomposition in I-SAB #1 was quite variable, indicating that the microbial community was not stable. In general, rates tended to decline
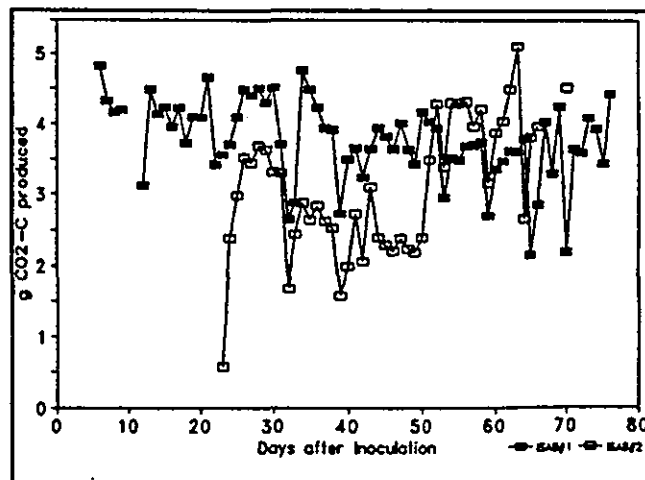

Figure 5. Time-courses of daily CO_2 production for I-SAB#1 and I-SAB#2 during the wheat grow-out experiment.

after ca. day 40. However, many of the abnormally high or low values could be explained by equipment or computer control problems. At the end of each run after crop growth requirements had been satisfied, the effect of air flow perturbation on various microbiological parameters was examined. These

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perturbations, which usually lasted for one day, are reflected in low values for carbon dioxide production as the bioreactor went anaerobic.

As shown in Table II, recovery of major cations and anions, i.e., nitrate, phosphate, potassium, calcium, and magnesium, from the wheat residues in the bioreactor effluent was comparable to aqueous extraction (leaching) results using the same wheat residue from BWT931. Effluent contained higher amounts of nitrate, probably due to pH control by addition of appropriate amounts of 1 N nitric acid. Bioreactor recovery of trace elements depended on the element. Iron recovery was poor and probably reflects immobilization by microbial cells, when compared with leachate. Recovery of copper and zinc was much better than for leachate.

Both leachate and bioreactor effluent mineral recovery systems use only inedible crop residues as starting material. The portion of minerals contained in edible biomass must also be recovered after passage through food processing and crew components of a CELSS. As a result, neither bioreactor effluent nor leachate were expected to recover all of the minerals needed to replenish a hydroponic solution.

For the wheat growth study on nutrient recycling, inorganic salts were added to the effluent or leachate, after results of inorganic analyses were known, to bring total concentration of each element to the same levels as were in the Hoagland's control replenishment solution. Results of the wheat growth tests showed that bioreactor effluent, so amended, could be used successfully to replenish the nutrient solution of hydroponically grown wheat (8).

SUMMARY AND CONCLUSIONS

Three Intermediate-Scale Aerobic Bioreactors were designed, fabricated and operated at the Kennedy Space Center's CELSS Breadboard Project. The bioreactors were successfully used to maintain, monitor, and control aerobic biodegradation experiments. These experiments focused on the characterization of the microbial mineralization process of inedible plant biomass, and the identification of optimum process parameters. Liquid effluent from the biodegradation process was shown to contain sufficient inorganic nutrient levels to support the hydroponic growth of candidate CELSS food-crops.

The I-SAB's are currently being used to further investigate aerobic degradation of inedible biomass. Bioreactor effluent is being used in long-term plant growth experiments that focus on the continuous production of white potatoes. In addition, I-SAB 3 has been modified to support anaerobic biodegradation studies and is currently being used for that purpose.

Results from the I-SAB aerobic processing experiments have been used to design a larger Breadboard-Scale Aerobic Bioreactor (B-SAB) that will be integrated with KSC's BPC to investigate large-scale recycling of inorganic nutrients. Protocols are also being developed to conduct fish feeding trials using harvested biomass from B-SAB.

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